The role of the gut microbiota in nutrition and health

Harry J. Flint, Karen P. Scott, Petra Louis and Sylvia H. Duncan

Abstract | The microbial communities that colonize different regions of the human gut influence many aspects of health. In the healthy state, they contribute nutrients and energy to the host via the fermentation of nondigestible dietary components in the large intestine, and a balance is maintained with the host's metabolism and immune system. Negative consequences, however, can include acting as sources of inflammation and infection, involvement in gastrointestinal diseases, and possible contributions to diabetes mellitus and obesity. Major progress has been made in defining some of the dominant members of the microbial community in the healthy large intestine, and in identifying their roles in gut metabolism. Furthermore, it has become clear that diet can have a major influence on microbial community composition both in the short and long term, which should open up new possibilities for health manipulation via diet. Achieving better definition of those dominant commensal bacteria, community profiles and system characteristics that produce stable gut communities beneficial to health is important. The extent of interindividual variation in microbiota composition within the population has also become apparent, and probably influences individual responses to drug administration and dietary manipulation. This Review considers the complex interplay between the gut microbiota, diet and health.

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Introduction

The relationship between the mammalian host and microorganisms that colonize the intestinal tract is the outcome of a lengthy and complex coevolution.[1] The primary imperative for the host must be to defend against the constant threat of infection that is posed by microorganisms in the gut. On the other hand, mammals have gained the ability to benefit from nutrients supplied by the resident microbiota, and the development of the gut and of the immune system is attuned to the presence of a complex microbiota.[2,3] Research into infectious diseases has always sought to identify single causative agents wherever possible, in most cases with remarkable success. Understanding the role of our gut microbiota in nutrition and the maintenance of health, however, represents a very different challenge that necessarily involves different approaches.[4] Certain organisms, such as bifidobacteria and *Faecalibacterium prausnitzii*,[5] are considered beneficial for health, although the supporting evidence and mechanistic basis for these benefits remain incomplete and in some cases equivocal. At the same time, the gut community also harbours organisms that have the capacity for adverse effects, via their metabolic outputs and gene products, or potential for pathogenicity.[6] The balance of benefit and harm for the host therefore depends on the overall state of the microbial community in terms of its distribution, diversity, species composition and metabolic outputs (Figure 1). In this Review, we focus mainly on the interplay between diet, the species composition of the microbial community and microbial metabolism in the healthy state.

Competing interests
The authors declare no competing interests.

The gut environment

The gut environment differs markedly between different anatomical regions in terms of physiology, digesta flow rates, substrate availability, host secretions, pH and oxygen tension. The human intestinal microbiota should therefore be viewed as a collection of semidiscrete communities. The large intestine, which is characterized by slow flow rates and neutral to mildly acidic pH, harbours by far the largest microbial community (dominated by obligate anaerobes) that will be the main subject of this article. Important differences in gut environment occur between proximal and distal regions, and more locally between the gut lumen and surfaces (Box 1; Figure 2). By comparison, the small intestine provides a more challenging environment for microbial colonizers given the fairly short transit times (3–5 h) and high bile concentrations.[7,8] Molecular analysis has revealed that the jejunal and ileal microbiota consists mainly of facultative anaerobes, including Gram-positive streptococci, lactobacilli and enterococci species and Gram-negative Proteobacteria and *Bacteroides*.[7,8] The major microbially produced short-chain fatty acids (SCFAs) detected in ileal effluents from individuals with an ileostomy were acetate, propionate and butyrate in the molar proportions of 20:1:4,[8] compared with approximately 3:1:1 in a typical faecal sample. Indications, however, exist that the

Microbiology group, Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK (**H. J. Flint, K. P. Scott, P. Louis, S. H. Duncan**).

Correspondence to: H. J. Flint
h.flint@abdn.ac.uk

Key points

- Molecular surveys have revealed remarkable diversity within the human gut microbiota, but certain dominant species are detected in faecal samples from most healthy adults
- Dietary intake, especially of nondigestible carbohydrates, alters the species composition of the gut microbiota both in the short term and in the long term
- Interindividual variation in colonic microbiota composition influences responses to dietary manipulation
- The gut microbiota potentially influences the host's energy balance through multiple mechanisms, including supplying energy from nondigestible dietary components and influences on gut transit, energy intake and energy expenditure
- Whether variation in gut microbiota composition is a major factor that influences obesity and metabolic disease in humans is not yet clear
- The latest research has suggested new candidate organisms among the healthy gut microbiota that might be beneficial to gut health and new strategies for correcting dysbiosis associated with certain disease states

microbiota of the intact terminal ileum might be different[9] and closer to that of the proximal colon.[10] Given the importance of the ileum as a site for interactions with the immune system and with pathogens, having more information on these communities is clearly desirable.

The 'normal' human colonic microbiota

Most of the information that is available on the composition of the gut microbiota derives from faecal samples that mainly reflect the community present in the lumen of the distal large intestine. Extensive analysis of small subunit (16S) ribosomal RNA (rRNA) sequences amplified from faecal samples[11–15] has been supplemented by data from metagenomic sequencing[16] to produce a broad consensus on microbial diversity; thus, the dominant bacterial phyla in the healthy state in humans are the Firmicutes, Bacteroidetes and Actinobacteria, with Proteobacteria and Verrucomicrobia also present in lower numbers.

Descriptions at a more detailed taxonomic level reveal many hundreds of species (or 'phylotypes', to include noncultured variation) in a typical faecal sample. These findings lead us to a series of important questions: to what extent can each individual be considered to carry a unique collection of gut microbiota (or, conversely, is there a 'core' set of gut bacteria that is common to everyone); to what extent do samples taken from the same individual vary in microbiota composition with time as a result of changes in diet, environment or other influences (for example, antibiotics); how much does gut microbiota composition change with life stage? Ideally, we need answers to all these questions in relation to the microbiota of the healthy gut before addressing the question of how microbiota changes might be associated with disease states. Fortunately, studies in the past few years have provided at least partial answers.

Dominant bacterial species in the colon

Despite the diversity at the level of phylotypes, it is clear that some species are commonly detected in high numbers in most adult faecal samples. Tap *et al.*[12] reported 66 particularly abundant phylotypes among 17 healthy individuals; it was noted that most of the same dominant phylotypes were common to those reported in

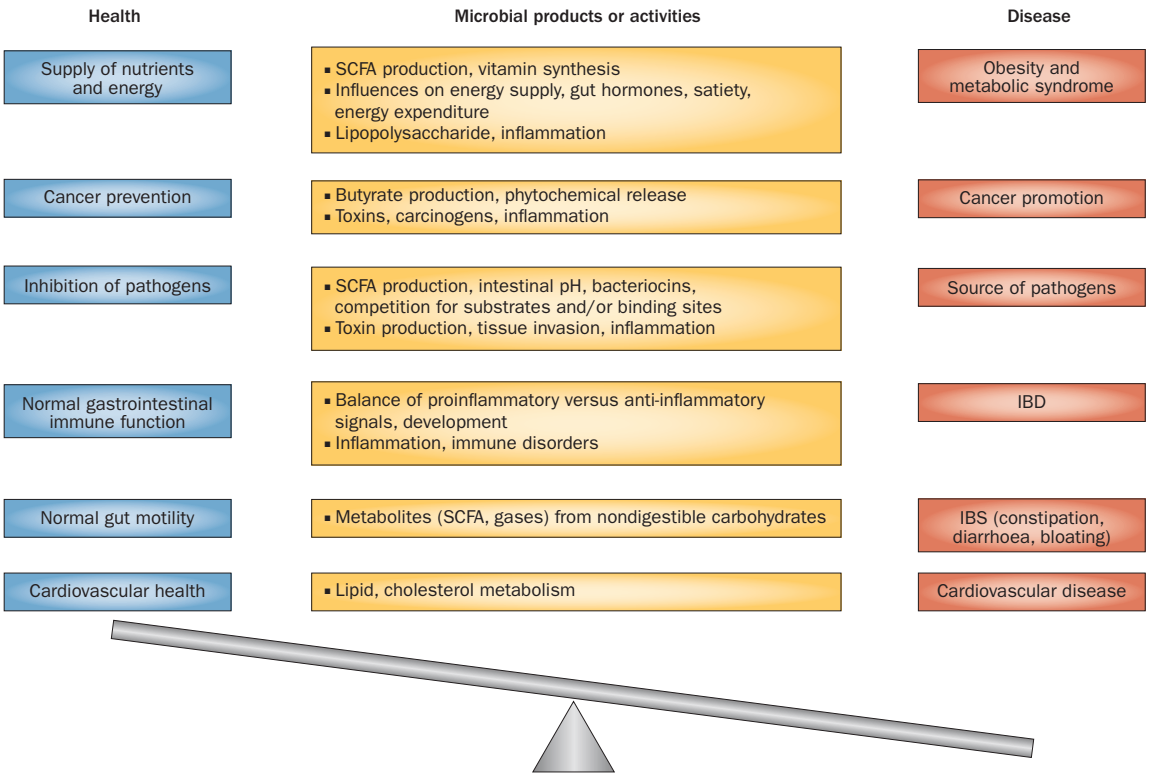

Figure 1 | Influence of gut microbial communities on health. Most of the microbial activities indicated in the centre column are functions of the whole community of gut microbiota rather than being attributable to a single species. The balance of the community and its output determines the net contribution to health or disease. Abbreviation: SCFA, short-chain fatty acid.

four previously published studies. Walker *et al.*[13] found 50 dominant phylotypes that each represented >0.5% of total 16S rRNA sequences across six obese male individuals; interestingly, 62% of these corresponded to cultured species, whereas only 28% of the remaining 270 phylotypes had cultured representatives. Five of the top 10 species (*Bacteroides vulgatus*, *Eubacterium rectale*, *F. prausnitzii*, *Colinsella aerofaciens* and *Ruminococcus bromii*; Figure 3) corresponded to the top five most abundant bacteria detected in an entirely culture-dependent study.[17] It should be no surprise that the most abundant phylotypes have been isolated preferentially, but this finding also suggests that the remaining microbial diversity might not have been cultured mainly because they are relatively less abundant and consequently occur sporadically among the most dominant bacteria in the community, rather than being intrinsically unculturable. Goodman *et al.*[18] also concluded that anaerobic culturing in principle enabled the recovery of the majority of diversity that was detected by sequencing at the species level in faecal samples. Evidence, however, indicates substantial geographical variation in dominant phylotypes,[19,20] suggesting that, for some human populations, many highly abundant species will not have been cultured. It should also be recognized that less abundant (or subdominant) species can have critical roles in the microbial community. Although much of this phylogenetic variation might be functionally redundant,[21] it could also include species that possess unique functional properties (for example, as 'keystone' species that release energy from recalcitrant substrates, or as pathogens) and that could contribute to major interindividual variation in health outcomes.

For practical reasons, most gut bacterial diversity is likely to remain uncultured and descriptions of the gut microbial communities will continue to depend almost entirely on rapid culture-independent molecular approaches. The arrival of high-throughput sequencing technologies offers the alternative approach of analysing the gene content of the community through metagenomics (Box 2) rather than focussing on phylogenetic groups.[16,20,22] Not surprisingly, as many core functions are conserved across species, gene content shows less interindividual variability than phylogenetic composition.[23] As the microbial cell is the basic unit of replication and metabolism, it still is essential to understand the coevolved collections of genes that represent individual genomes, together with the interactions of individual microbes with each other, and with the host.

Phylogenetic analyses have demonstrated marked variation in the phylotypes present between individuals within populations.[13,24,25] Large-scale sequence analysis has also suggested that the human intestinal community might exist in a small number of discrete states or 'enterotypes',[26,27] although the degree of discontinuity for interindividual variation within the human microbiota is not yet clear.[28]

Impact of diet upon the gut microbiota

Faecal microbiota profiles in healthy adults seem to have substantial stability over time.[29–31] The influence of particular dietary components can, however, be seen in carefully controlled human dietary studies. Although many studies have documented the response of selected groups to prebiotics,[32] only a few have examined temporal changes in the whole gut microbial community in response to dietary change (Table 1). In one 2011 study in which obese male volunteers were given controlled diets differing in the type and content of nondigestible carbohydrates for 3-week periods, faecal microbiota profiles tended to group by individual more than by diet.[13] On the other hand, there were marked changes in the relative abundance of several dominant phylotypes in response to the dietary shifts, especially increased intake of resistant starch (Figure 3). These changes occurred within a few days, and were reversed equally quickly by a subsequent dietary switch. The species affected were mainly those already shown to utilize starch, but the same species did not respond in the same manner in all individuals. In other studies, supplementation with galacto-oligosaccharides or inulin was shown to increase the relative abundance of bifidobacteria on average, but again certain volunteers were found to be 'nonresponders'.[33,34] Thus, changes in the intake of nondigestible carbohydrates clearly affect faecal microbiota composition, but these responses are not universal and are influenced by the initial composition of an individual's gut microbiota. It should also be noted that many dominant groups of bacteria, perhaps those that possess a greater degree of nutritional diversity or flexibility, remained unaffected by dietary change.

Box 1 | Influence of the colonic environment upon gut microbiota composition

Intestinal pH gradients

Colon pH varies from mildly acidic conditions in the proximal colon to more neutral pH distally. Growth of *Bacteroides* spp. is curtailed by pH values <6.0 at short-chain fatty acid concentrations typical of the colon (50–100 mM).[89] Many Firmicutes are more tolerant of acidic pH, giving them a competitive advantage at the low pH that can result from active substrate fermentation. A major shift in species composition and metabolic outputs of the human intestinal microbiota has been seen between pH 5.5 and pH 6.5 in a continuous culture model *in vitro*.[60]

Intestinal oxygen gradients

Another factor that influences the spatial distribution of the microbiota is oxygen.[144] The colonic lumen becomes highly anaerobic (Eh of ~250 mV) largely because facultative anaerobes consume available oxygen. Most colonic bacteria are obligate anaerobes that fail to grow $>5 \times 10^{-3}$ atm oxygen, but *Bacteroides* spp. can be 'nanaerobes'; *B. fragilis* possesses a cytochrome *bd* oxidase that allows growth in nanomolar oxygen concentrations.[145] Although most colonic Firmicutes are considered strict anaerobes that become inviable in air within minutes,[58] growth of *Faecalibacterium prausnitzii* is actually stimulated by very low oxygen concentrations because of its ability to shuttle electrons to oxygen via flavins and thiols,[83] which suggests that *F. prausnitzii*, like *B. fragilis*, might exploit niches close to the mucosa that involve some exposure to oxygen.[144]

Bile acids

Bile acids are derived from cholesterol in the liver and secreted as conjugated bile acids into the small intestine, followed by deconjugation by microbial bile salt hydrolases.[146] Reabsorption in the small intestine also contributes to gradients of bile acid concentration. Bile acids have strong antimicrobial activity; feeding cholic acid to rats caused a major shift in gut microbiota composition towards Firmicutes and against Bacteroidetes.[147] In the large intestine, bile acids are modified by the gut microbiota via 7-α-dehydroxylation to form potentially carcinogenic secondary bile acids.[99]

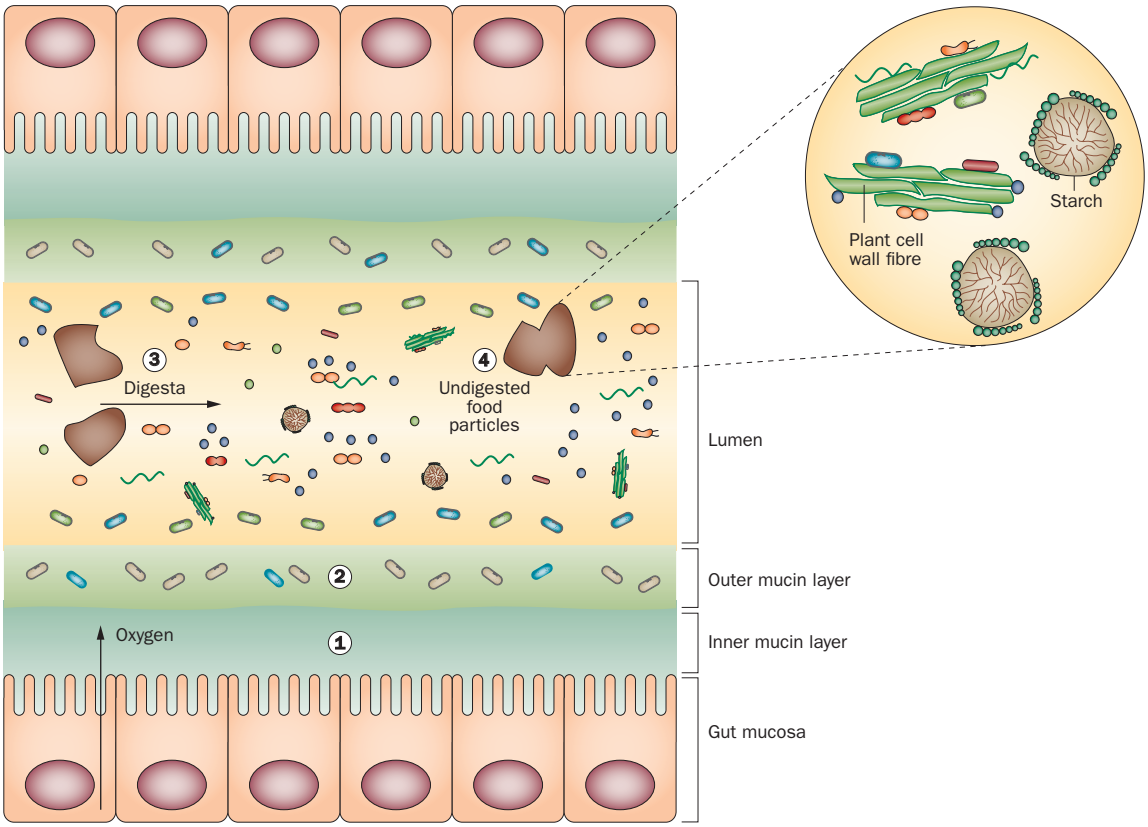

Figure 2 | Microbial microenvironments within the large intestine. Several microenvironments exist within the large intestine in which microorganisms can reside: 1) epithelial surface and inner mucin layer (minimal colonization in the healthy state); 2) diffuse mucin layer (specialist colonizers, for example, *Akkermansia muciniphila*); 3) gut lumen–liquid phase (diverse microbial community); and 4) gut lumen–substrate particles (specialized primary colonizers e.g. *Ruminococcus* spp.).

Interestingly, a correlation was reported in 2011 by Wu *et al.*[27] between two enterotypes defined in 96 adults and long-term dietary habits. Thus, a '*Prevotella*-type' community was associated with fibre intake and a '*Bacteroides*-type' community with high protein intake, suggesting that enterotypes might reflect discrete patterns of habitual dietary intake within the study population. An earlier study by de Fillippo *et al.*[19] detected major differences in faecal microbiota between Italian and African children, which they ascribed to differences in dietary intake. 16S rRNA sequences corresponding to *Prevotella* spp. were more abundant in the African children than in the Italian children and their overall intake of vegetable fibre was higher than in the Italian children. The Italian children showed higher proportions of *Bacteroides* spp. and Firmicutes than the African children, together with higher intakes of starch and protein. This finding suggests that, in addition to short-term changes induced by dietary shifts, long-term consequences of habitual diet upon the composition of the gut microbiota exist; although less evidence is available, it seems possible that such long-term changes would also be reversible by dietary change.

Development of the gut microbiota

The composition of the microbiota changes substantially at three stages in life: from birth to weaning; from weaning to attaining a 'normal' diet; during old age. The first bacteria to colonize the gut at birth are facultative anaerobes;[35] these bacteria in turn create anaerobic conditions that promote the growth of obligate anaerobes (initially *Bifidobacterium* and *Bacteroides* spp.) within about 2 weeks. Infants born naturally become inoculated by the mother's vaginal and faecal microbiota during birth,[36] although those born by caesarian section are initially colonized by bacteria from the environment and skin.[37] At 3 days, naturally delivered newborn babies harbour a greater abundance and variety of *Bifidobacterium* spp. than those born by caesarian section.[38,39] Babies that are solely breastfed until weaning tend to have a more stable, less diverse, bacterial community,[40,41] with higher proportions of bifidobacteria than formula-fed babies.[40,42,43] Studies using PCR amplification and sequencing[44] have not always reflected the abundance of *Bifidobacterium* spp. reported in other studies, although improved primers for 16S rRNA gene amplification are now available.[45] An early metagenomic study found that the faecal microbiota in Japanese infants was distinctly different to that of weaned children and adults, and that 80% of the infant sequences matched *Bifidobacterium*-derived sequences.[22] Some evidence indicates geographical variation in the composition of the gut microbiota, with bifidobacteria dominating in northern Europe and *Bacteroides* spp. and lactobacilli in

southern Europe,[40] although bifidobacteria dominated in breastfed babies both in Malawi and Finland.[46] Bacterial strains found in breast milk have also been detected in faecal samples from the corresponding babies.[47,48] These bacteria are postulated to translocate from the mother's intestine to the mammary gland via the mesenteric lymph nodes.[49] The early gut microbiota in preterm infants was reported to be dominated by facultative anaerobes;[50] this finding might be, in part, because of the necessary medical interventions involved in preterm birth, including the administration of antibiotics.

After the introduction of solid food, gut microbiota composition develops towards the adult pattern with increased diversity[43,51] and increased abundance of anaerobic Firmicutes.[52] The microbiota of breastfed and formula-fed babies converge gradually, becoming indistinguishable by around 18 months of age,[43] and resembles that of an adult by age 3 years.[20] Changes in the genetic capacity of the microbiome with human development include changes in the abundance of genes involved in vitamin biosynthesis.[20] Early colonization of the gut has been shown to influence maturation of the immune system,[53] and there might be a link between aberrant gut microbiota and atopic diseases such as eczema.[54,55]

A decline in microbiota diversity has been reported in old age,[56] with reduced numbers of bifidobacteria and an increase in Enterobacteriaceae.[57] Bacteroidetes become more abundant and Firmicutes less abundant in elderly adults (aged >65 years) compared with younger adults (28–46 years) as controls.[25] How these changes correspond to changes in health status is not yet clear, as is to what extent they are driven by altered dietary intake, physical activity or altered immune function.

Microbial metabolism in the gut

The metabolic activities of gut microorganisms have major consequences for the host that can be both beneficial and harmful. Metabolism in anaerobic microbial communities is highly interactive, with crossfeeding between different organisms an important and widespread phenomenon.[58] In a few cases, it is possible to associate particular metabolic products with one, or a few, species, as with the conversion of oxalate by *Oxalobacter formigenes*.[59] The situation is generally far more complex, with particular metabolites being produced by many members of the microbial community and being consumed or transformed by others. Crucially, although the species composition of the microbiota clearly has a role,[60] it is the substrates that are available to the microbiota that largely determine the metabolic outputs from the community.[61] As discussed earlier, dietary substrates have a major influence on the species composition of the microbiota, but their structures also determine which metabolic pathways are used for fermentation by individual bacterial species.

Dietary nondigestible polysaccharides

Much of the undigested dietary residue that arrives in the large intestine is in the form of insoluble particles (especially plant cell walls and resistant starch).[62]

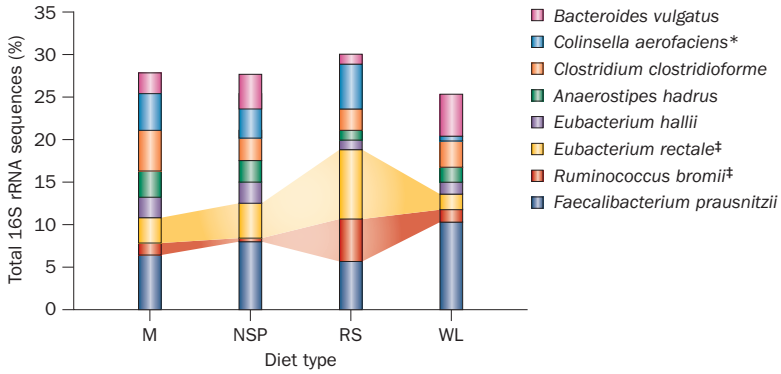

Figure 3 | Influence of diet upon dominant human colonic bacteria determined by 16S rRNA gene sequencing. The graph shown is based on data from means for six obese male volunteers in a controlled dietary trial reported by Walker *et al*.[13] Weight maintenance diets were M (control); NSP, high in wheat bran (3 weeks); RS, high in type 3 resistant starch (3 weeks); and WL, high in protein and reduced in carbohydrates (3 weeks). Data are from faecal samples analysed at the end of each dietary period. Statistically significant differences in the percentage abundance of a given species compared with the other diets are indicated (*P<0.05, ‡P<0.001). The eight most abundant bacterial species shown here together accounted for 29% of the total 16S rRNA sequences detected in these samples. Abbreviations: M, maintenance; NSP, nonstarch polysaccharide; RS, resistant starch; WL, weight loss.

Evidence from *in vitro* model systems suggests that specialized groups of bacteria are involved in accessing these structures.[63] In 2012, Ze *et al.*[64] presented evidence that *R. bromii*, species belonging to the Firmicutes, might act as a key primary degrader of resistant starch particles in the human colon, making the substrate available to other amylolytic bacteria. Analysis of faecal samples from healthy volunteers had previously revealed a markedly higher proportion of Ruminococcaceae sequences associated with the particulate fraction (12.2%) than with the liquid fraction (3.3%),[65] whereas the Gram-negative *Bacteroides* sequences tended to partition more with the liquid phase. This finding suggests that certain bacteria are preferentially associated with insoluble digesta in the gut and might represent specialist primary degraders of these substrates. Although many human colonic *Bacteroides* spp. are found to possess large genomes extremely rich in diverse glycoside hydrolase genes,[66] these bacteria seem better equipped to utilize soluble rather than insoluble carbohydrates.[67] The parts played by different human gut bacteria in carbohydrate breakdown are only now beginning to be understood.[68]

Utilization of host-derived substrates

Mucin provides a protective barrier for the gut epithelium, but is also a potential growth substrate for intestinal bacteria.[69] The specialized mucin-degrader *Akkermansia muciniphila* is an important member of the healthy colonic microbiota that has been found to modulate immune responses in a mouse model.[69,70] Other colonic species, notably *Bacteroides* spp., have the ability to utilize a variety of host-derived glycans.[66,68]

Short-chain fatty acid metabolism

Under the anaerobic conditions of the large intestine, undigested carbohydrates are fermented mainly to SCFAs

Box 2 | 'Meta-omics' analysis of gut microbial communities

High-throughput metagenomic sequencing potentially provides information on the full complement of functional genes in the microbial community, in contrast with the phylogenetic information that is obtained from amplification of ribosomal RNA genes.[16] The currently available technologies can already yield several gigabases of sequence per sample, and throughput is expected to increase further. Rather less attention has been paid to metagenomic approaches that target specific functional genes, although these enable in-depth analysis of functional groups within the microbiota at lower cost and without the need for massive bioinformatic capability. Phylotypes detected using such a targeted approach to amplify β-glucuronidase genes from human faecal DNA were found to correlate well with a large metagenomic dataset.[108] Another example of this approach targeted the butyryl-CoA:acetate CoA-transferase gene for butyrate formation;[21] 88% of sequences from 12 healthy volunteers were closely related to 12 cultured isolates, whilst only 12% belonged to novel uncultured phylotypes, suggesting that the dominant butyrate-producing bacteria are well represented by cultured species. Degenerate primers have also been developed for functional genes of hydrogenotrophic microbial groups, acetogens, sulphate-reducing bacteria and methanogenic Archaea[148–150] and for genes involved in xylan breakdown.[151] There is a clear need for more functional analysis, however, as a major proportion of genes identified during nontargeted metagenomic analyses remain of unknown function. Screening of metagenomic libraries provides one approach for detecting novel genes with specific functions of interest,[152] but the availability of genome sequences for cultured isolates of human gut bacteria that enable functions to be confirmed—for example, by gene knockouts—remains crucial. '–Omics' approaches can also be used to reveal those gene products that are most highly expressed within particular gut environments, either at the RNA (metatranscriptomic) or protein (metaproteomic) level.[153] Metatranscriptomics has been applied to patients with an ileostomy to gain information on the small intestinal community.[8]

(such as butyrate and acetate) and gases (hydrogen, carbon dioxide, methane and hydrogen sulphide). SCFAs have multiple effects on the host, as the major anions in the colon and as energy sources for the host, with butyrate being consumed mainly by the colonic epithelium and acetate becoming available systemically.[71] It has also been recognized that SCFAs signal to the gut receptors free fatty acid receptor 2 (FFAR2, formerly known as GPR43) and free fatty acid receptor 3 (FFAR3, formerly known as GPR41).[72] These receptors are involved in controlling anorectic hormones—including peptide YY (PYY) and glucagon-like peptide 1 (GLP1)—that have roles in appetite control, thus providing a potential link between microbial SCFA formation and food intake.[72] Other reported influences include anticancer effects (especially for butyrate), anti-inflammatory properties[73,74] and changes in gut motility[75,76] and energy expenditure.[77] Therefore, changes in the relative production rates of the major SCFAs by the colonic microbiota are likely to have important physiological consequences.

Considerable progress has been made in defining the dominant groups of bacteria that have key roles in anaerobic metabolism on the basis of cultured isolates, 16S rRNA-based molecular detection and new approaches targeted at particular functionally relevant genes[61] (Box 2). Two butyrate-producing Firmicutes *F. prausnitzii* and *Eubacterium rectale*, for example, are among the most abundant bacteria in the healthy colonic community.[13,21] Although these two species use similar routes for butyrate synthesis, relying on butyryl CoA:acetate CoA transferase,[78] evidence indicates that they have distinct ecological niches. *E. rectale* and the closely related *Roseburia* spp. are flagellated bacteria with the ability to utilize a range of dietary polysaccharides, especially starch.[79–81] *F. prausnitzii* is nonflagellated and fails to utilize many dietary polysaccharides, including starch.[82] Furthermore, the stimulation of *F. prausnitzii* by low concentrations of oxygen (Box 1) was not observed for the close *E. rectale* relative *Roseburia inulinivorans*.[83] In human volunteer trials, weight-loss diets low in total carbohydrate have been shown to decrease the percentage of butyrate among faecal SCFAs,[84,85] correlating with decreased populations of the butyrate-producing *Roseburia* plus *E. rectale* group.[84,86] By contrast, *F. prausnitzii* showed little change in its representation among the faecal microbiota with low-carbohydrate diets.[13,84] Faecal butyrate concentrations have been shown to increase with total SCFA concentrations under conditions of more rapid gut transit,[75,87,88] an effect that might be mediated partly via the influence of pH on the gut community[60,89] (Box 1).

Acids such as lactate, succinate and formate normally behave as intermediates in microbial metabolism in the gut because of onward conversion (Figure 4). Certain Firmicutes that are dominant in the healthy colon, *Eubacterium hallii* and *Anaerostipes* spp., have the ability to convert lactate and acetate into butyrate.[90] *E. hallii* numbers were shown to increase in faecal slurries in the presence of lactate *in vitro*,[91] and substantial flow of label has been noted from ^{13}C lactate to butyrate in the mixed community in isotope labelling studies to monitor SCFA production.[92–94] Label was also found in propionate in these studies, assumed to be due to conversion of lactate to propionate by members of the Veillonellaceae. Given the widespread formation of lactate and its low pKa (acid dissociation constant), the activities of these lactate-utilizing bacteria are likely to have an important role in maintaining homeostasis within the community. Lactate can accumulate under conditions of disturbance (or dysbiosis)—for example in severe colitis[95]—probably due to the curtailment of the growth of lactate-utilizing bacteria at reduced pH.[91] Less widely recognized is that acetate, although almost invariably reaching the highest concentration among faecal SCFAs, is also an intermediate that is consumed by the major butyrate-producing bacteria[78] (Figure 4).

Although most sugars are fermented by common or converging pathways to yield SCFAs,[96] some require alternative routes. The deoxyhexose sugars fucose and rhamnose, for example, are fermented by some intestinal bacteria via propanediol to yield propionate and propanol.[97] Propionate production from hexose sugars is thought to be mainly associated with the Bacteroidetes and the Veillonellaceae (Firmicutes); most seem to use the succinate pathway for propionate formation, with only a few bacterial species known to use the acrylate pathway.[61]

Fermentation of amino acids derived from dietary or host-derived proteins yields a much wider range of products.[98] Faecal branched-chain fatty acids are indicative of fermentation of branched-chain amino acids and their faecal concentrations increase on high-protein diets.[86]

Table 1 | Diet-driven changes in gut microbial community composition in humans*

Dietary intervention	Duration (weeks)‡	Volunteers§	Molecular profiling methods (16S rRNA)	Bacterial changes detected	Reference
Controlled diet composition					
Resistant starch (RS3)	3	14 obese, M	Sequencing; qPCR	↑ *Ruminococcus bromii*, *Eubacterium rectale*, *Roseburia* spp. and *Oscillibacter* spp.	Walker *et al.* (2011)[13]
Nonstarch polysaccharides (wheat bran)	3	14 obese, M	Sequencing; qPCR	No major changes	Walker *et al.* (2011)[13]
Weight-loss diet‖	3	14 obese, M	Sequencing; qPCR	↓ *Collinsella aerofaciens*, *E. rectale* and *Roseburia* spp.	Walker *et al.* (2011)[13]
Weight-loss diets‖	4	18 obese, M	FISH	↓ *E. rectale*, *Roseburia* spp. and *Bifidobacterium* spp.	Duncan *et al.* (2007)[84]
Weight-loss diets‖	4	17 obese, M	FISH	↓ *E. rectale*, *Roseburia* and *Bifidobacterium* spp.	Russell *et al.* (2011)[86]
Dietary supplementation					
Resistant starch (RS2)	3	10 healthy	Sequencing; qPCR	↑ *R. bromii* and *E. rectale*	Martínez *et al.* (2010)[154]
Resistant starch (RS4)	3	10 healthy	Sequencing; qPCR	↑ *Bifidobacterium* spp. and *Parabacteroides distasonis*	Martínez *et al.* (2010)[154]
Resistant starch (Hi Maize)	4	46 healthy	DGGE; qPCR	↑ *R. bromii*	Abell *et al.* (2008)[155]
Inulin and oligofructose	2.3	12 healthy	qPCR	↑ *Faecalibacterium prausnitzii* and *Bifidobacterium* spp.	Ramirez-Farias *et al.* (2009)[34]
Inulin (long chain)	3	31 healthy	FISH	↑ *Bifidobacterium* spp., *Lactobacilli* spp. and *Atopobium* spp. ↓ *Bacteroides* spp. and/or *Prevotella* spp.	Costabile *et al.* (2010)[156]
Inulin	2	30 healthy	FISH	↑ *Bifidobacterium* spp. ↓ *Bacteroides* and/or *Prevotella* and *Clostridium histolyticum*	Kleessen *et al.* (2007)[157]
Galacto-oligosaccharides	3	18 healthy	Sequencing	↑ *F. prausnitzii* and *Bifidobacterium* spp. ↓ *Bacteroides*	Davis *et al.* (2011)[33]
Raffinose	3	12 healthy	Sequencing; qPCR	↑ *F. prausnitzii*, *Bifidobacterium* spp.	Fernando *et al.* (2010)[158]

*Recent studies (within previous 5 years) that have attempted to analyse the whole gut microbe community (from faecal samples) and provide detailed information on dietary intake are shown. Many additional studies have shown stimulation of specific groups, especially bifidobacteria (reviewed[32]). ‡Most studies employed a crossover design comparing the influence of different diets within individuals. Duration refers to a single dietary period. §Adults of both sexes unless otherwise stated (M = males only). ‖High protein, reduced levels of carbohydrates. Abbreviations: DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence *in situ* hybridization; qPCR, quantitative PCR.

Potentially toxic or carcinogenic products of protein fermentation include *N*-nitroso compounds, amines and cresol.[99]

Hydrogen disposal

Hydrogen has a key role in anaerobic ecosystems. Its disposal reduces the levels of gaseous compounds produced in the colon and also affects the metabolism of hydrogen-producing fermentative bacteria by enabling a shift in the relative production of different fermentation products.[100] Which hydrogenotrophic microbial group dominates potentially also has important health effects. Hydrogen sulphide generated by sulphate-reducing bacteria (SRB) is generally regarded as a toxic product,[101] although it is also produced by the human body and influences host functions, including promoting the healing of ulcers and anti-inflammatory effects.[102] Methanogenesis is associated with a slower transit time,[87] which might reflect the slow growth of methanogenic Archaea that use hydrogen and carbon dioxide, or formate, to form methane. Intriguingly, evidence exists that methane might actively be involved in prolonging intestinal transit.[103] Acetogenic bacteria can produce acetate from hydrogen and carbon dioxide, or formate, as well as from carbohydrates.[104]

A 2012 study investigating human biopsy samples from the left and right side of the colon and the rectum of 25 healthy individuals found that, using PCR primers that target functional genes, all individuals carried all three groups of hydrogenotrophs, with methanogenic Archaea comprising around 50% of hydrogen utilizers in each of the three regions of the colon. SRB were more abundant than acetogens in the right colon and acetogens more abundant in the left colon and rectum.[105] SRB are able to use lactate as a cometabolite to produce hydrogen sulphide and acetate[106] (Figure 4).

Metabolism of phytochemicals and xenobiotics

Colonic bacteria are also involved in the release and transformation of a wide range of non-nutrient, but potentially bioactive, compounds of plant origin, including a wide variety of aromatic compounds.[107] Some of these derive from the degradation of plant-cell-wall structures. For example, ferulic acid, an important component of cereal bran, is largely converted to 4-OH phenylpropionic acid in faecal samples.[86] Other aromatic

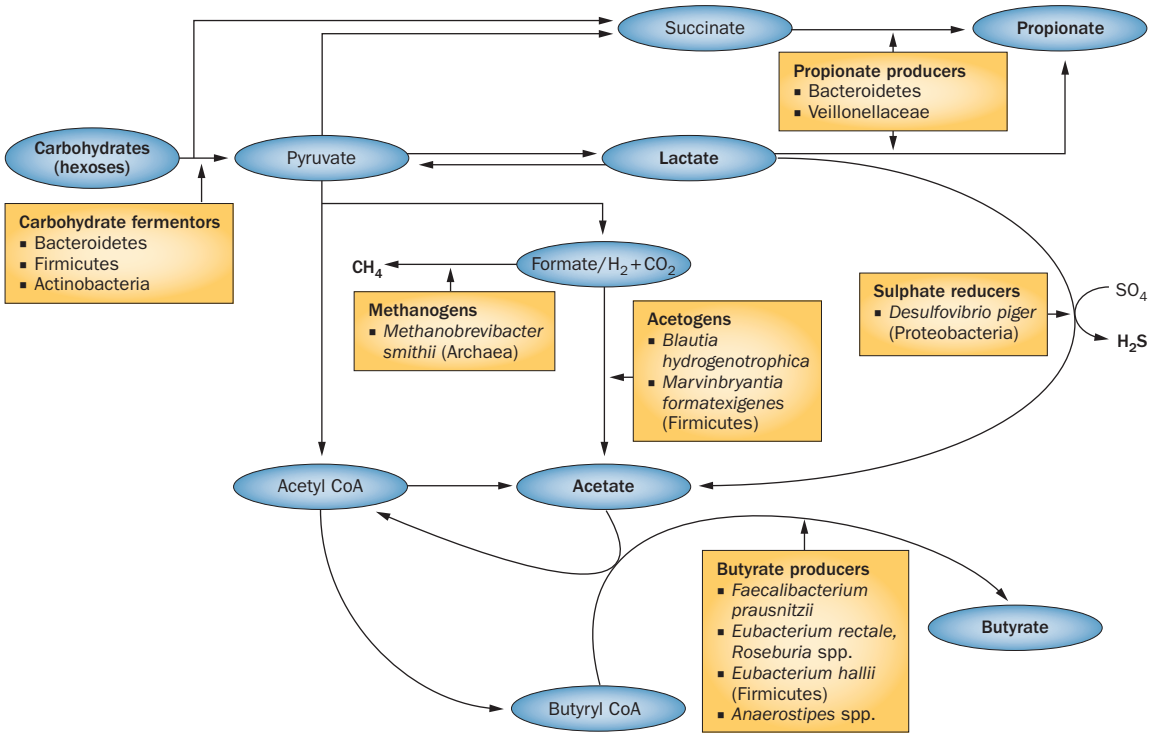

Figure 4 | Functional and phylogenetic groups of gut bacteria involved in the metabolism of short-chain fatty acids. Figure shows a schematic of the gut microbiota involved in the metabolism of short-chain fatty acids. Acetate and lactate are shown as intermediates. Representative species and phyla are indicated based on information from cultured microorganisms. Whereas most colonic bacteria use the Embden–Meyerfhof pathway for hexose metabolism, bifidobacteria (Actinobacteria) use the bifid shunt pathway (not shown here).[96]

compounds derive from hydrolysis of soluble glycoside conjugates present in the plant.[107]

Many plant compounds, and also drugs, are treated as xenobiotics and conjugated to form glucuronides in the liver, then to be released into the gut; these glucuronides are subject to hydrolysis by microbial β-glucuronidase, releasing the original compound. A targeted analysis has shown that a fairly small number of bacterial phylotypes account for most copies of the bacterial *gus* (β-glucuronidase) gene found present in the human colon.[108] Thus, variation in the populations of these few species would be expected to result in considerable inter-individual variation in *gus* activity and the cleavage of glucuronide conjugates in the gut. Although a possible second gene responsible for β-glucuronidase activity has been identified in human gut bacteria,[109] its contribution is not yet clear.[108] A wide range of metabolites formed by microbial activity in the gut can be detected in the bloodstream, and a number of these metabolites have potential as biomarkers of health or disease.[110]

Energy, obesity and metabolic health

As noted above, absorption of microbially produced SCFAs provides energy to the host from dietary components that have remained undigested in the small intestine. Gut microbes, therefore, contribute to the 'energy harvest' from the diet,[111] and this contribution might be vital under conditions of food scarcity. The microbial contribution to the host's energy supply will depend on many factors, including the nondigestible carbohydrate content of the diet and upon gut transit, which affects SCFA absorption and the extent of digestion and fermentation of dietary carbohydrates.[112,113] The species composition of the gut microbiota also has the potential to influence energy harvest; for example, through variation in keystone species responsible for the breakdown of recalcitrant substrates[64] or the proportions of different metabolic groups involved in forming SCFAs or gaseous products. The calorific value per mole of nondigestible carbohydrate is considerably less than for a fully digestible carbohydrate (Figure 5) and clearly depends on the extent of fermentation and of SCFA absorption.[114] This finding means that directly replacing digestible carbohydrate by nondigestible carbohydrate in the diet should reduce the net delivery of calories to the host, assuming equal intake. Some evidence indicates that dietary nondigestible carbohydrate might contribute to satiety.[115]

Much speculation has been made over the possible contribution of gut bacteria to obesity in humans, initially focussing on the possible influence of microbiota composition on energy harvest. The balance of evidence does not show a consistent phylum level shift in microbiota composition in obese humans.[13,116–118] Although a lower *Bacteroides*:Firmicutes ratio was reported in obese individuals in one human study and in *ob/ob* mice,[24,111] other human studies have reported the opposite result,[116] or no difference.[117] More subtle changes might occur in

obese individuals at the species level, but these changes could be the result either of different dietary habits[84] or altered host physiology. Changes in faecal microbiota profile (towards Bacteroidetes) have been reported for individuals with type 2 diabetes mellitus.[119]

Small animal studies continue to suggest intriguing, but complex, links between gut microbiota composition and adiposity.[120] Germ-free rodents can show markedly greater, or lesser, gains in adiposity and body weight than conventional animals when fed high-fat diets.[121,122] These different outcomes depend on the exact composition of the diet supplied and seem to correlate with influences on energy expenditure more than energy harvest (these diets in any case contain little fibre).[122] A number of studies have demonstrated a major influence of high-fat diets on microbiota composition in rodents.[123,124] Nevertheless, a series of studies involving transfer of gut microbiota from obese animals to germ-free lean animals have suggested that the composition of the gut microbiota can influence adiposity, with concomitant changes in either energy harvest, energy intake or energy expenditure.[111,125,126] Evidence has also been obtained from small animal studies that increased passage of bacterial lipopolysaccharide (LPS) into the bloodstream occurs during consumption of high-fat diets. It has been proposed that this increase in LPS might be involved in the development of insulin resistance that is triggered by high-fat diets.[127] Proinflammatory LPS is assumed to originate mainly from Gram-negative Proteobacteria in the small intestine.

Intestinal health

Substantial evidence exists for modification of the faecal and colonic microbiota in certain forms of IBD, especially ileal Crohn's disease,[128,129] which are discussed in detail elsewhere in this Focus issue.[130] Some Firmicutes, notably *F. prausnitzii*, are reported to show decreased representation in patients with ileal Crohn's disease. Furthermore, patients with low *F. prausnitzii* abundance had a greater likelihood of relapse following surgical resection than those with higher abundance.[5] Combined with evidence that *F. prausnitzii* produces an anti-inflammatory product, this finding has led to strong interest in this organism as a potentially beneficial component of the healthy gut microbiota. It has also been noted that patients can recover despite having very low numbers of *F. prausnitzii*,[131] and it remains to be established whether *F. prausnitzii* is simply an indicator of the gut environment or an agent that actively influences gut health. The contribution of potentially pathogenic Proteobacteria that are suspected to have a key role in causation of IBD has been reviewed in detail.[132]

In the case of IBS, several studies have found that imbalance in the gut microbiota can be detected in dominant bacterial ribotypes[133] or in functional groups.[134] As yet, no clear consensus exists on the changes that occur in different forms of IBS or their clinical significance in terms of aetiology.[135] In general, it can be difficult to disassociate the effects of the active disease state and of treatment regimes upon the microbiota from

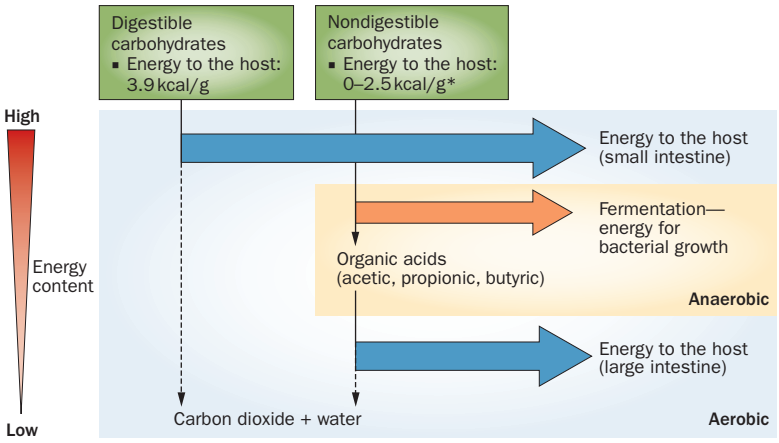

Figure 5 | Contribution of ingested carbohydrates to dietary energy supply to the host. Fermentation of dietary substrates by anaerobic microorganisms in the large intestine enables the recovery of only a fraction of the initial energy content for microbial growth. This step allows the host to absorb and oxidize the SCFAs that are produced as microbial fermentation products. The energy yield to the host from nondigestible carbohydrates through this route will vary depending on the efficiencies of fermentation and of absorption of the SCFA products. *Estimates from Roberfroid study.[114] Abbreviation: SCFA, short-chain fatty acid.

compositional changes that might be causative or protective. Studies that include patients at first presentation or in remission can, therefore, provide valuable insights.

The gut microbiota is considered to have an important role in the prevention of sporadic colorectal cancer through the production of butyrate and the transformation of certain dietary phenolics.[99] On the other hand, cancer-promoting compounds can also be generated by microbial activity, and the balance of procarcinogenic and anticarcinogenic actions is highly dependent on diet and xenobiotic intake.[136] Bacterial changes have been noted between patients with cancer and healthy control groups; a 2012 study based on next-generation sequencing found, amongst other changes, a decrease in butyrate-producers in patients with colorectal cancer,[137] but, again, the causal relationship between microbiota profile and cancer development remains unclear.

Some gut pathogens seem able to modify host responses and the gut environment—so as to favour their own proliferation[138]—by producing major changes (dysbiosis) in the resident gut microbial community. Little is known about how gut microbial communities recover following episodes of diarrhoea, or indeed after antibiotic treatment.[139]

Future perspectives

The latest reports indicate that replacement of disturbed gut microbiota by faecal microbiota from a healthy individual can be a highly successful approach to treating *Clostridium difficile* infection.[140–143] This approach is also being considered for other disease states in which the causation is less well understood, including gut disorders such as IBD and IBS, and autoimmune diseases.[143] In future, the idea of developing a restorative 'cocktail' of beneficial bacteria normally present in the healthy colonic microbiota might look increasingly attractive as

an alternative to using somewhat undefined faecal preparations. Furthermore, evidence showing that the composition of the gut microbiota responds to diet indicates that prebiotic approaches for delivering health benefits can be made more targeted and effective. These goals will require a far more detailed characterization of key members of the healthy gut community and their interactions with each other, and with the host, than is currently available. Such information would also assist greatly in interpreting the large metagenomic datasets that are currently being produced with the aim of understanding the role of the gut microbiota in the aetiology of human diseases. The combination of gut microbiology, gastroenterology and epidemiology with developments in the rapid analysis of metabolites, microbial markers and molecular signals promises exciting progress in the coming years.

Conclusions

Molecular analyses have revealed remarkable diversity within the colonic microbiota of adult humans. Although certain dominant bacterial species are detected in most healthy adults, there is also substantial interindividual variation in microbial community composition. Dietary intake determines the metabolic outputs of the microbial community at the same time as modifying the species composition. Diet, therefore, offers a potential route to delivering health benefits through manipulation of the microbial community. It remains challenging, however, to define those states of the community that are most beneficial to health and those that pose a long-term risk to health. Achieving this feat will depend both on more detailed functional analysis of representative cultured species, and on information from new '-omics' approaches that examine shifts in overall gene complement and expression within the community.

Review criteria

Multiple literature searches were made using Web of Science and Scopus (1950–2012) using as search terms: "gut bacteria/microbiota/microflora/flora" AND, for example, "bile, phytochemicals, metabolites, obesity OR infant". The broad scope of this article, together with limitations on length, however meant that not all papers could be cited. The resulting selection inevitably represents the authors' own assessment of relevance to the topic, with some bias towards recent papers. Where recent comprehensive reviews were already available on topics of current interest (for example, bacteriotherapy, microbiota involvement in IBD and IBS) we have provided these rather than reporting the primary literature.

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